REVISTA CIENCIAS BIOMÉDICAS

ARTÍCULO ORIGINAL

Exploring cross reactivity potential of DER F 24, a cytochrome allergen from *Dermatophagoides Farinae*: a bioinformatic approach

Explorando el potencial de reactividad cruzada de DER F 24, un alérgeno de citocromo de Dermatophagoides Farinae: un enfoque bioinformático

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ACCESO ABIERTO

Para citaciones: Buendía, E., Garcés, J., Chávez, Y., Escobar, M., Sánchez, A., & Múnera, M. (2024 Exploring cross reactivity potential of DER F 24, a cytochrome allergen from *Dermatophagoides Farinae*: a bioinformatic approach. Revista Ciencias Biomédicas, 13(2), 58-64. https://doi.org/10.32997/rcb-2024-4801

Recibido: 5 de diciembre de 2023
Aprobado: 1 de abril de 2024

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Editor: Inés Benedetti. Universidad de Cartagena-Colombia.

ABSTRACT

Introduction: Der f 24, which is a *Dermatophagoides farinae* characterized allergen, is a ubiquinol cytochrome c reductase binding protein (UQCRB) homolog. Experimental data revealed that IgE reactivity on Der f 24 is concentrated on an epitope located in amino acid positions 1-32, corresponding to the N-terminal region. However, potential cross reactivity between Der f 24 and other allergenic sources have not been explored using experimental or *in-silico* approaches.

Objective: In this study, using previous published experimental data and bioinformatic tools we explored potential Der f 24 cross-reacting allergens across various important allergenic sources in the tropics.

Methods: Multi alignment among amino acid sequences of Der f 24 and common allergenic sources (crustaceans, insects, mites, rodents, helminths, and Bos taurus) was performed to explore identity and structural homology. ElliPro and BepiPred in silico tools were used to predict B cell epitopes. Consurf tool was used to conduct identification of conserved regions among homologues.

Results: Were found twelve homologous for Der f 24 in various allergenic sources such as mites, insects, crustaceans, and mammals, averaging 65% homology among them. Three linear epitopes (15-19 GFRK, 48-51 RRLP and 75-80 FLPKEQW) and a discontinuous epitope were predicted (K105, K107, E108, E109, I112, N113), all of them conserved among UQCRB studied here. Finally, according to ConSurf analysis, epitopes predicted in this study are highly conserved among the UQCRB protein family.

Conclusions: Was found cross reactivity between two Der f 24 and various homologous allergenic sources such as mites, insects, and mammals, suggesting Der f 24 is an allergen with high cross reactivity potential.

Keywords: Allergen; epitope; cross-reactivity; bioinformatic; IgE antibodies.

RESUMEN

Introducción: Der f 24, que es un alérgeno caracterizado de *Dermatophagoides farinae*, es un homólogo de la proteína de unión de ubiquinol citocromo c reductasa (UQCRB). Datos experimentales revelaron que la reactividad de IgE en Der f 24 se concentra en un epítopo ubicado en las posiciones de aminoácidos 1-32, correspondiente a la región N-terminal. Sin embargo, la posible reactividad cruzada entre Der f 24 y otras fuentes alergénicas no ha sido explorada utilizando enfoques experimentales o *in silico*.

Objetivo: en este estudio, utilizando datos experimentales previamente publicados y herramientas bioinformáticas, exploramos posibles alérgenos que reaccionan de manera cruzada con Der f 24 en diversas fuentes alergénicas importantes en los trópicos.

Métodos: se realizó una alineación múltiple entre las secuencias de aminoácidos de Der f 24 y fuentes alergénicas comunes (crustáceos, insectos, ácaros, roedores, helmintos y *Bos taurus*) para explorar la identidad y la homología estructural. Se utilizaron las herramientas in silico ElliPro y BepiPred para predecir epítopos de células B. La herramienta Consurf se utilizó para identificar regiones conservadas entre homólogos.

Resultados: se encontraron doce homólogos de Der f 24 en varias fuentes alergénicas como ácaros, insectos, crustáceos y mamíferos, con un promedio de 65% de homología entre ellos. Se predijeron tres epítopos lineales (15-19 GFRK, 48-51 RRLP y 75-80 FLPKEQW) y un epítopo discontinuo (K105, K107, E108, E109, I112, N113), todos ellos conservados entre las UQCRB estudiadas aquí. Finalmente, según el análisis de ConSurf, los epítopos predichos en este estudio están altamente conservados entre la familia de proteínas UQCRB.

Conclusión: se encontró reactividad cruzada entre Der f 24 y varios homólogos en fuentes alergénicas como ácaros, insectos y mamíferos, lo que sugiere que Der f 24 es un alérgeno con alto potencial de reactividad cruzada.

Palabras Clave: Alérgeno; epítopo; reactividad cruzada; bioinformática; anticuerpos IgE.

INTRODUCTION

Allergy to mites is a worldwide public health concern, due to allergic symptoms triggered by exposure to mites' allergens in day-to-day modern society. Species such as *Blomia tropicalis, Dermatophagoides pteronyssinus* and *Dermatophagoides farinae* are important allergenic sources (1) and several allergens have been characterized, including: tropomyosin, paramyosin, α-tubulins, serine-proteases and fatty acid binding proteins (1). Mite's allergens cross react with allergens present in other sources such as: insects, helminths, and crustaceans, being tropomyosin the main allergen responsible for this cross reactivity (2-4). This phenomenon is important to allergology, because the cross sensitization and co-exposure can exacerbate allergic symptoms and make the diagnosis of allergy to a determined allergenic source (5, 6). In such a way, the characterization of allergens that cross react with mites will help to improve the diagnosis and management of allergic diseases.

The traditional study of cross reactivity usually involves the characterization of IgE binding specificities to the studied allergenic source, demonstration of the IgE inhibition by the suspected cross-reacting allergens and finally epitope mapping to determine the epitopes involved in the cross reactivity phenomena (7). However, other strategies can be used as the initial approach to study cross reactivity narrowing the candidates for cross-reactive epitope mapping. For example, bioinformatic approaches have been validated to predict cross reactive epitopes on allergens (8-10) and computational methods can be used to calculate the Property Distance Scale or the comparison of antigenic surfaces on 3D structures of potential cross-reactive allergens (11, 12).

Der f 24, is an allergen from *D. farinae* that has been previously characterized (13), it is an ubiquinol-cytochrome c reductase binding protein (UQCRB) homolog and experimental data revealed that IgE reactivity on Der f 24 is concentrated in an epitope located in amino acid positions 1-32, corresponding to N-terminal region (13). However, potential cross reactivity of Der f 24 with other allergenic sources have not been explored experimentally or even in-silico. In this study, using previous experimental data and bioinformatic tools we explored homologs in other allergenic sources and the potential allergens that could cross react with Der f 24, the cross-reactive epitopes and its conservation across homologs.

METHODS

Antigens and allergens retrieve

Aminoacidic sequence of Der f 24 was retrieved from the Uniprot database with accession number: M9RZ95. PSI.BLAST was performed using Der f 24 sequence as input. Search was limited to common allergenic sources (crustaceans, insects, mites, rodents, helminths, and Bos taurus), also, a homologous species in human was used for comparisons (P14927). General parameters were set up by default. Amino Acid sequences from bacteria with clinical relevance for human were used for further analysis.

Multiple alignment analysis

IBIVU PRALINE tool was used to perform multiple alignment of amino acid sequences among Der f 24 and homologous (8). BLOSUM62 was used as an Exchange weights matrix; other parameters were set up as default.

Modeling based on homology

All 3D structures of Der f 24 and homologous were determined by modelling based on homology using Swiss Model server (9). UCSF chimera allowed to perform root median square deviation (RMSD) analysis, and Pymol software was used to visualize models (10, 11). Root Median Deviation Square values were determined using a matchmaker tool reported in Chimera.

Evolutionary analysis

Consurf tool (12) was used to calculate evolutionary conservation of Der f 24, and identify regions conserved that could help to explain molecular mimicry among human Der f 24 and homologous. Algorithm HMMER and one iteration with an E-value cutoff of 0.0001 were used as default parameters.

Epitope prediction

Ellipro and Bepipred servers were used to predict epitopes on human Der f 24 (13, 14). Epitopes with conserved amino acid residues among homologous and Der f 24 were reported. We used experimental data from epitope mapping reported on literature (13).

RESULTS

Multialignment analysis and structural analysis

In total, twelve homologs to Der f 24 were found in several allergenic sources. Multiple alignment showed a 65% in identity level among Der f 24 and

all the UQCRB examined. Excluding residues from 1 to 20 on alignment, all aminoacid sequences showed a high conservation level (Figure 1). For example, residues position 49 to 56, 78 to 89 and 96 to 104. All ubiquinol-cytochrome c reductase binding proteins modeled showed a typical folding of this kind of protein family, consisting of four alpha helices connected between them with short loops (Figure 2). Superpositions revealed that Der f 24 shared high structural homology with the found homologous, RMSD analysis for some structural superpositions was 0.22 (Der f 24 and UQCRB from *D. pteronyssinus*) (Figure 3) (Table 1).

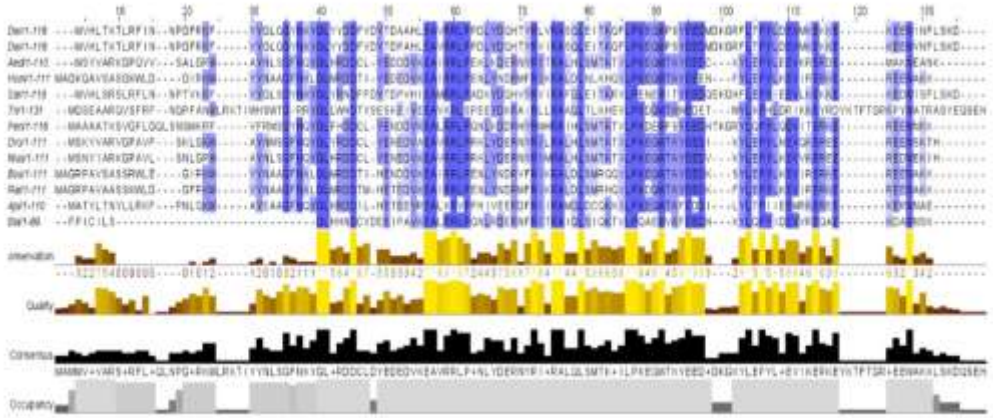

Figure 1. Multiple alignment shows conservation (65%) among aminoacid sequences derived from homologous to Der f 24 reported in common allergenic sources. Alignment was performed with Jalview software.

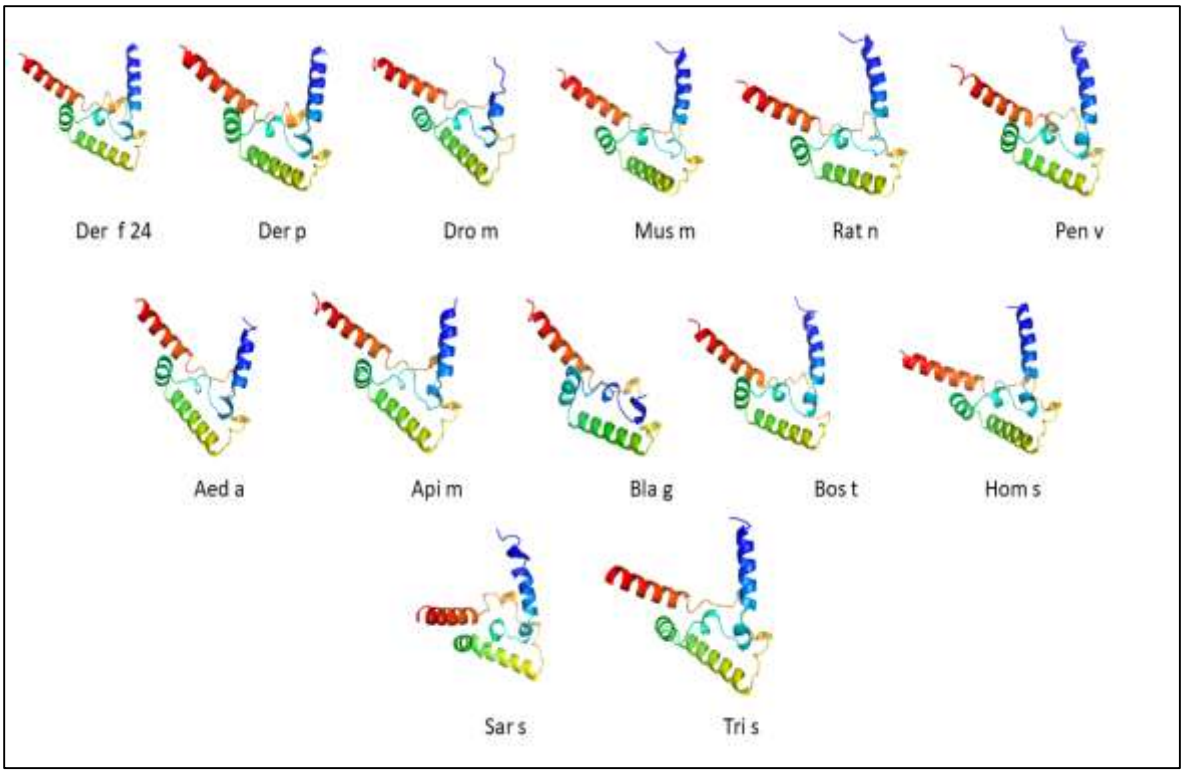

Figure 2. Tridimensional models obtained by modeling based on homology for all homologous to Der f 24, 3D models showed a typical folding of UQCRB.

Biological sources	Access code (Uniprot)	ERRAT (Overall Quality Factor)	RMSD
Dermatophagoides farinae	M9RZ95	97.9	-
Dermatophagoides pteronyssinus	A0A6P6XTE8	95.9	0.22
Drosophila melanogaster	Q9VAW7	98.8	0.7
Mus Musculus	Q9CQB4	79.5	0.6
Rattus norvegicus	B2RYS2	79.5	0.55
Penaeus vannamei	A0A3R7MKT6	96	0.7
Aedes aegypti	Q5MM88	100	0.78
Apis mellifera	A0A088ASD9	100	0.6
Blattella germanica	PSN32986.1	97.4	0.62
Bos taurus	P00129	88.6	0.8
Homo sapiens	P14927	88.6	0.84
Sarcoptes scabiei	A0A132A7I2	94.1	0.2
Trichinella spiralis	A0A0V1BE49	89.6	0.45

OBJ

Table 1. The quality values (ERRAT) obtained by the 3D models obtained by homology are shown. In general, the models showed adequate folding. The RMSD values obtained from the superposition of the 3D structure of each homologue to Der f 24 are also shown.

Epitope prediction and evolutionary analysis

Using experimental data about epitope mapping referred to (13), we found that residue positions 1-32 described in the literature was conserved in Der f 24 and the other UQCRB modeled structures from residue 21 to 32 (Figure 1). Epitope prediction showed that this region was not involved in cross reactivity, but three linear epitopes (15-19 GFRK, 48-51 RRLP and 75-80 FLPKEQW) and a discontinuous epitope were predicted (K105, K107, E108, E109, I112, N113) outside the previously reported immunodominant epitope (13). Finally, all these epitopes were conserved among UQCRB studied here, according to multiple alignment (Figure 3) and Consurf analysis (Figure 4b and c).

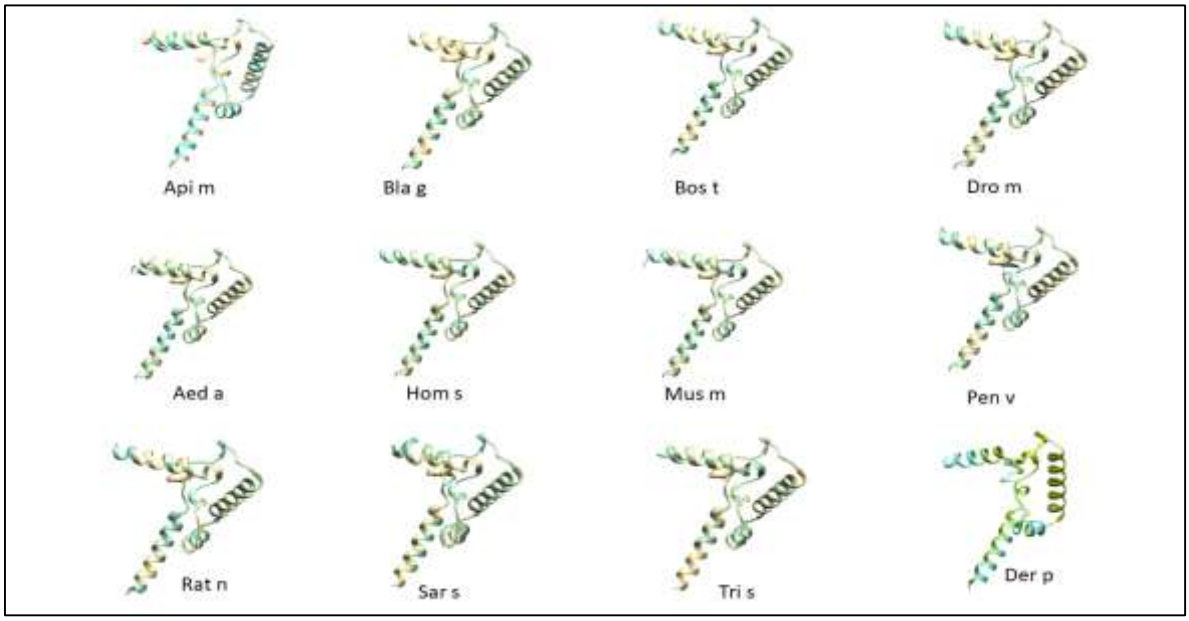

Figure 3. Superposition between Der f 24 (Blue) and each homologous model (Brown). All RMSD values were calculated using Chimera software. Some overlapping Der f 24 and homologous from *D. pteronyssinus* exhibited a value of 0.22, suggesting high structural homology.

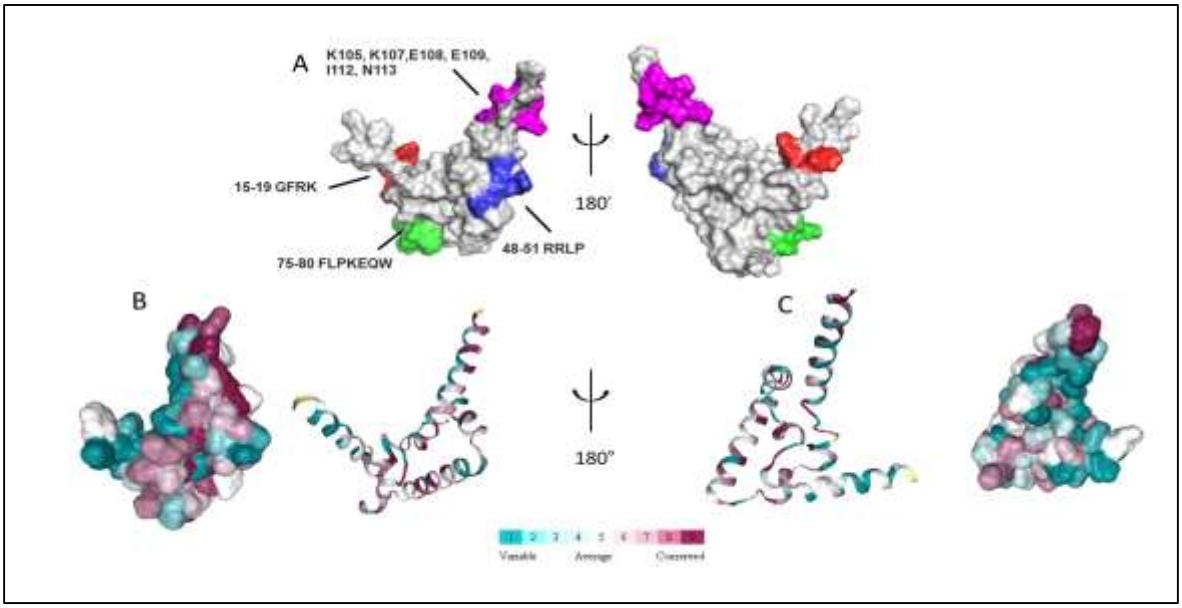

Figure 4. Models from Der f 24. A: surface model showing epitopes conservated among Der f 24 and all homologous. B and C: Consurf models indicating evolutionary conservation in UQCRB. According to this, epitope conformed by residues K105, K107, E108, E109, I112, N113 is in a conservatory high region. This supports the fact that Der f 24 is an allergen with potential capacity to be cross reactive.

DISCUSSION

Der f 24, is an allergen from *D. farinae* that has been previously characterized (13), it is an ubiquinol-cytochrome c reductase binding protein (UQCRB) homolog and experimental data revealed that IgE reactivity for Der f 24 is concentrated in an epitope located in the positions 1-32, corresponding to the N-terminal region (13). Here we found twelve homologs for Der f 24 in other allergenic sources such as mites, insects, and mammals, averaging 65% homology among them. Three lineal and six discontinuous epitopes were predicted for the whole allergen. Importantly, two of the lineal and all the discontinuous epitopes were located outside the immunodominant IgE epitope reported in the literature and were highly conserved.

UQCRB is a crucial protein involved in mitochondrial energetic respiratory chain present across all eukaryotes, making it an excellent pan-allergen candidate since ubiquitously present (14) and potentially cross-reactive. For pan-allergens such as tropomyosin, homology averaged 56%-98% with other cross-reactive tropomyosin from shrimp, lobster, house dust mites (HDM) and cockroaches (4, 15). In our study we reported a 65% homology for the Der p 24 and UQCRB from other sources, and various highly conserved epitopes. Structurally, we found epitopes located outside the immunodominant epitope reported for Der f 24 so far (13), which is an important finding of our in-silico approach. It is pending to establish the importance of those epitopes in tropical regions with simultaneous co-exposure to all the potentially cross-reacting sources. In conclusion, Der f 24 is an allergen with high potential to be cross reactivity with other important allergenic sources. This could help to understand cross sensitization in allergic subjects.

CONTRIBUTIONS OF THE AUTHORS: all the authors participated in conception and design of the study, data collection, analysis and interpretation, drafting of the article, critical review and approval of the final version, and are responsible for the veracity and integrity of the article.

CONFLICTS OF INTEREST: the authors declare no conflicts of interest for the conduct and publication of this study.

FUNDING: the present research did not receive specific grants from public, commercial or non-profit agencies.

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